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Microbial means of biowaste management

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Abstract:

The rise in the municipal, agricultural and industrial waste is posing threat to the ecology and environment by polluting our land, water and air. The gaseous discharge from waste heaps contributes to global warming. Hence proper waste management is the need of the hour. Waste can be degraded through incineration, landfills, charcolization, or microbial decomposition. On an average ~ 80% of the waste consists of organic material, which can be microbiologically degraded to produce fertilizers (compost) for agricultural use. Thus, the paper reviews the microbial means of biowaste management through aerobic and anaerobic digestion and the role of various groups of microbes in these systems. Both these processes is being considered as possible routes for treatment of the biodegradable fraction of municipal and other solid wastes and it is anticipated that their use will become more popular over the years.

INTRODUCTION

Waste generation is an integral part of human society. A huge amount of waste is generated during the course of production, distribution and consumption of food. Municipal solid waste (MSW) consists of everyday items like wastepaper, kitchen waste, clothes, packaging materials, bottles, electrical appliances, etc. On an average, organic wastes constitute 80 per cent of MSW, which is biodegradable. If the amount of organic agricultural waste, such as corn stalks, leaves and wheat straw from wheat-processing facilities, sawdust and other residues from wood mills, is also considered, this component of solid waste could be a principal resource for biodevelopment [1]. In Japan, garbage accounts for 1% (by weight) of total industrial waste and includes residues generated during industrial food-manufacturing processes. However, it accounts for 34% of total general waste and includes residues generated during cooking in restaurants, feeding facilities, and homes, as well as food beyond its expiry date and food refuse [2]. Household garbage is gradually increasing due to greater use of disposable materials by the society, which in turn is imposing extra burden on the environment, particularly in the urban areas.

Waste, if not properly managed can lead to serious health and environmental risks. Abiotic and biotic conversion of solid wastes (renewable carbon sources) generated by the municipal, agricultural, forestry, industrial and manufacturing sectors of the economy cause large-scale pollution of land, water and air. Uncontrolled release of the gaseous products of waste decomposition into the atmosphere contributes to global warming [3].

Garbage (biodegradable fraction of waste) accounts for around 1% (by weight) of total industrial waste and includes residues generated during industrial food-manufacturing processes. However, it accounts for 34% of total general waste and includes residues generated during cooking in restaurants, feeding facilities, and homes, as well as food beyond its expiry date and food refuse [4].

In most developing countries, the degradable organic matter from wastes is dumped in the open which undergoes aerobic or anaerobic degradation. These unengineered dumpsites permit fine organic matter to become mixed with percolating water to form leachate which may pollute adjoining water and soil [5]. With the increasing need to conserve natural resources and energy, recycling of organic wastes assumes major importance.

Waste can be degraded either through physicochemical methods such as incineration, landfills, charcoalization, dehydration, etc. or through microbial decomposition by means of aerobic or anaerobic digestion.

Landfills are engineered areas in which waste is buried in the land with various systems and safeguards to prevent ground water contamination, but the waste still poses health risks because it generates certain greenhouse gases [6]. In general, landfill gases consist of five principal components *viz.* methane, carbon dioxide, hydrogen,

oxygen and nitrogen. Trace compound gases, such as saturated and unsaturated hydrocarbons, acidic hydrocarbons, organic alcohols, aromatic hydrocarbons, sulfur compounds and inorganic compounds, are also released from municipal landfill sites [7].

Incineration is another method of managing solid waste and is popular in countries with a severe shortage of landfill sites. It is described as the controlled combustion of waste with the primary purpose of reducing the combustible portion of it and of recovering the available energy from the process. This controversial process is portrayed as the worst way of dealing with biowaste because it destroys resources for biorecycling or composting. Recycling of waste proves to be an effective management option because it does not involve the emission of many greenhouse gases and water pollutants. This approach saves energy, supplies valuable raw materials to industry, stimulates the development of green technologies, conserves natural resources and reduces the need for new landfill sites and incinerators [8].

Other physicochemical approaches to garbage treatment include charcoalization and dehydration, both of which are, at present, very minor methods for the garbage treatment. In the charcoalization method, garbage is charred at high temperatures (for example, 250~1000^{0}C) under oxygen-free or oxygen deficient conditions and the resultant product can be used as fuel, adsorbent, and soil conditioner. However, this method is not cost-effective, and the formation of a by-product, tar, during the process remains problematic. In the dehydration method, garbage is dehydrated at lower temperatures (less than 100^{0}C), and the product is used as fertilizer. The main drawback of this method is that the product can easily be rehydrated due to humidity and rainfall, which may cause putrefaction of the material during storage. Moreover, the dehydrated material is not better as a fertilizer than the compost that is produced through microbial processes [4].

Microbial means of biowaste management, which is the topic of this review, utilizes the microorganisms to degrade organic waste to produce compost, animal feeds, food products for example fish sauces or lactic acid through aerobic digestion (composting) or biogas (methane or biogas) through anaerobic digestion which can be used as fuels for cooking or lighting. Microbial methods share several desirable features that are in striking contrast to those of physicochemical methods as follows, and are expected to play more important roles in the future recycling of garbage. First, microbial methods are versatile; namely, different types of products may be obtained depending on the method employed and the nature of garbage to be processed. This allows site- or community-specific approaches to the recycling of garbage. Second, microbial methods can be performed with low energy consumption compared with other methods. Third, unlike in the case of incineration and landfill, only negligible amounts of environmental pollutants are produced through microbial methods. Finally, products generated through microbial methods can be treated in accordance with the global cycling of materials, as typified by composting and animal feed production. Thus, recycling of garbage by means of microbial methods is expected to have far less impact upon the natural environment compared with the other methods [4].

Although microbial methods share the promising features outlined above, each has specific problems, some of which will be mentioned below. It is important to note here that the characteristics and problems of the respective methods are closely related to the microbiological characteristics of the process. The types of microorganisms that are involved in the microbial degradation process depend largely upon the methods employed, the nature of the garbage, and other environmental factors. Analyses of microflora and its transition during the course of such processes are of great interest from the viewpoint of microbial ecology. Moreover, the analyses should provide important clues that help to overcome the problems with microbial methods for recycling garbage. Unfortunately, only incomplete information is available regarding such analyses performed using classical culture- based methods, because a great majority (99%<) of microbial inhabitants are only viable in the ecological niche of the processes and appear to be unculturable or culture-to-difficult under laboratory conditions [9]. However, recent progress in molecular biological approaches to analyze microflora in natural environments has provided a break- through to enhance our understanding of the microbiological aspects of the microbial degradation of garbage [4].

Although microbiological treatment methods are considered to be best, we know very little regarding the microbiology involved in such treatment methods. However, much progress has been made over the past few decades regarding determination of types of microorganisms existing in certain system using molecular microbial ecological approaches [10].

These microbial processes of waste management reduces environmental burden and yields useful bioproducts from waste which plays a key role in achieving sustainability in agricultural production because it possesses many desirable properties such as high water holding capacity, cation exchange capacity (CEC), ability to sequester contaminants (both organic and inorganic) and beneficial effects on the physical, chemical and biological characteristics of soil. The organic degradable refuse of plant and animal origin provides a good source of nutrients to improve soil productivity.

AEROBIC DIGESTION

Aerobic digestion is the process of degradation of organic waste into a humus-like stable product using aerobic microorganisms under moist and self-heating conditions. The method in which aerobic digestion is employed for conversion of biowaste into useful products is popularly called composting.

Composting has been defined as a controlled-microbial aerobic digestion process with the formation of stabilized organic materials, which may be used as soil conditioners and/or organic fertilizers [10-14]. The history of composting in a broad sense started in ancient times when Greeks and Romans used organic wastes that had rotted for long times [15]. Animal and food wastes were composted before being used as fertilizers in the early civilizations of South America, China and India. The myth that composting is a "natural process" that can be left to itself has impeded the development of composting for a long time [16]. Until industrial times, the focus was directed towards the uses of compost, and little interest was shown in process control criteria. Research on composting seems to have begun in the 1980s with one of the earliest publications appearing in the United States where results from agricultural experimental station were presented [15]. Since then, the number of composting facilities has increased greatly, especially during 1980s and 1990s. The increase in waste generation, concomitant with the increase in the economic growth has been a major incitement for the establishment of the composting plants [17].

As the landfill directive 99/31/EC sets stringent requirements for the diversion of the biodegradable fraction of municipal waste from the landfill, composting of green waste is increasingly regarded as an attractive option for the partial achievement of this target [18]. However, for composting to have any future as an important component of an integrated waste management system its product, the compost, should be able to find its way to the market, a prerequisite that could only be achieved if compost quality is quantifiable and can be measured and certified though appropriate assurance systems. Compost stability constitutes an important, and probably the most controversial, aspect of overall compost quality in terms of definition and evaluation [19]. In this context, it is important to better understand the dynamics of the process and assess the rate and the degree of the organic matter decomposition (compost stability), in order to facilitate the design of efficient systems and produce composts that may find their way into the markets [19, 20].

The organic waste is a mixture of several compounds. Despite the heterogeneous mixture of organic source materials it can be divided into following major elements - carbohydrates, proteins, fats, hemicellulose, cellulose, lignin and mineral matter [15]. The first three groups of organic matter, which are very susceptible to decomposition, include compounds like sugars, starches, pectin, fatty acid, lipids, amino acids and nucleic acids. Hemicellulose, cellulose and lignin, on the other hand, are much more resistant to decomposition, and mineral matter is mainly unaffected by the process [17].

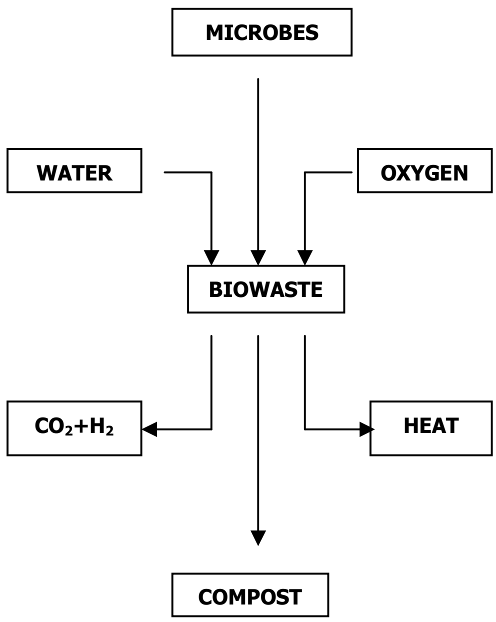

Fig. Basic concept of composting process
(based on Epstein, 1997)

Many factors determine the existence of various microbial communities during composting. Temperature is the major factor that determines the types of

microorganisms, species diversity, and the rate of metabolic activities [21]. During composting, a great deal of energy is released in form of heat in oxidation of the carbon to CO_2. For example, if a gram-molecule of glucose is dissimilated under aerobic conditions, 484 to 674 kilogram calories (kcal) of heat may be released. If the organic material is in a pile or is otherwise arranged to provide some insulation, the temperature of the material during decomposition rises to over 170°F. However, if the temperature exceeds 162°F to 172°F, bacterial activity decreases and stabilization is slowed down.

Oxygen is another essential component necessary for metabolic activities of aerobic microorganisms in a composting pit. Oxygen is supplied by active mechanical aeration, convective air-flow (= passive aeration), and physical turning of the compost mass [15]. Oxygen diffusion can be limited by moisture content exceeding 60%, because the free pore space may be blocked by water [22]. Excessively wet compost material becomes anaerobic, which inhibits the growth of aerobic microorganisms. Low moisture content, on the other hand, can also limit microbial activity, because water is the essential medium, in which nutrients diffuse and availability of the nutrients may become limited [17].

During composting carbon dioxide and water is released as decomposition products. Respiratory carbon dioxide is evolved during microbial activity, and the change in carbon dioxide emissions reflects the metabolic activity during the composting process. In the beginning, readily available carbon is utilized and released carbon dioxide increases to a peak almost simultaneously alongwith peaks of temperature and released moisture [23]. As the process continues, however, the rate of carbon dioxide evolution decreases as the availability of carbon decreases, leading to decrease in metabolic activities.

Based on the development of temperature, the composting process can be divided into the mesophilic phase (temperatures below 45^0C), the thermophilic phase (temperatures above 45^0C), and finally the curing phase, which is characterized by a decrease in temperature [17]. The temperature usually rises to 50^0C during the process, and the maximum desirable composting temperature is considered to be 60^0C, based on microbial species diversity and the rate of decomposition [24]. The temperature rise is important for public health, as pathogens in the compost are destroyed during the thermophilic composting process [21, 25,26].

Essentially, the microorganisms decompose the organic matter into a stable amendment for improving soil quality and fertility [27, 28]. The success of the composting process, however, relies on the ability of the microbial community to sustain with its basic needs for moisture, oxygen, temperature control, and nutrient availability [15]. A typical composting process goes through a series of phases, including a rapid temperature increase, sustained high temperatures and a gradual cooling of the composting mass. Different microbial communities dominate during these various composting phases, each adapted to a particular environment [29]. It is important to mention that a large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms, including bacteria, actinomycetes, yeasts and various other fungi have been extensively reported in composts and other self-heating organic materials [30-37]. The initial stage of composting is characterized by the growth and activity of mesophilic organisms such as, fungi, yeast, gram negative and lactic acid bacteria [16,29,30,38]. In the next phase, thermophilic temperatures are reached, as a result of the activities of the mesophilic community, and organisms adapted to these conditions such as *Bacillus* spp., *Thermus thermophillus*, and *Thermoactinomyces* spp. takes over the degradation process [24,34,39,40]. The growth and activity of non-thermotolerant organisms, including pathogens and parasites, are inhibited during the thermophilic stage. During this stage, the pH stabilizes in a range of 7.5-8.5 [41], because of the consumption of organic acids and mineralization of nitrogen [42]. The final curing stage is characterized by the development of a new mesophilic community. As the temperature declines, mesophiles and moderate thermophiles reappear including fungi and actinomycetes [30,43]. The later group of microorganisms is nowadays referred to as Actinobacteria, probably to emphasize that they are not fungi, but belong to the Gram-positive bacteria [17]. Actinobacteria are commonly believed to play a significant role in the degradation of relatively complex, recalcitrant compounds [44]. The ability of Actinobacteria to degrade lignocellulose implies that this group of bacteria may be suitable as potential indicator organisms for compost maturity [17].

Compost stability is an important aspect of compost quality, determining compost nuisance potential, nitrogen immobilization and leaching and phytotoxicity [19,45]. If insufficiently stabilized compost is applied to land, it can create anaerobic conditions in the rhizophere or induce phytotoxicity, mainly caused by organic acids present during the early stages of composting process [46]. Compost is sufficiently stabilized when the rate of

oxygen consumption is reduced to the point in which anaerobic or malodorous conditions are not created to an extent that they interfere with the storage, marketing and use of the end product. In addition, stabilized composts should not have problems with vermin attraction, pathogen re-growth or other problems resulting from its incomplete decomposition [22]. Inspite of its importance, there is still no universally accepted parameter for the determination of compost stability and a wide range of physical, chemical and biological parameters have been proposed. The most promising methods seem to be those based on compost microbial activity, usually measured through the respiration activity (e.g. AT_4, Dynamic Respiration Index, SOUR) or the self-heating potential, which are increasingly being described in compost quality standards [19,45,47].

The utilization of biowaste relieves stresses on the environment, but it should not be ignored that a variety of heavy metals and pathogens can be found in raw biowastes [48,49]. The total content of heavy metals in biowastes destined for agricultural use is of primary importance. Concern regarding the heavy metal pollution of agricultural soils is related essentially to crop quality and human health. The amount of these elements should not reach thresholds, which may either damage soil fertility (toxic effect against microorganisms, inhibition of mineralization and humification, perturbation of bio-geochemical cycles) or the food chain (heavy metals uptake by plants, crop species cultivated and ingested by the human or animals) [48].

On the other hand, the composting process can, if not properly managed, induce the proliferation and dispersion of potentially pathogenic and/or allergenic thermotolerant/thermophilic fungi and bacteria [37,50]. Among the fungi, the mold *Aspergillus fumigatus* is predominant because of its cellulolytic and thermotolerant properties [37]. Among the bacteria, *Salomonella*, *Shigella*, *Escherichia coli*, *Enterobacter*, *Yersinia*, Streptococci and *Klebsiella* can emerge and cause infection for compost handlers and agricultural users [49].
Although composting is an old and familiar technology, the composting process is one of the most complex biotechnologies because of the myriad physical and biological states during the process. Garbage degradation could be partly explained by the succession and function of predominant strains detected by culture-independent methods. However, a global understanding of the composting process would require the analysis of the whole microbial community including those microorganisms present in low numbers and barely detectable (< 0.1 % of total microorganisms). In the present century, the microbiology of composting should be studied from various aspects, for example, composition, succession, microhabitat, and function of microorganisms within the community, because composting is considered as one of the most important techniques for the reutilization of abundant organic waste [2].

ANAEROBIC DIGESTION

During anaerobic digestion, organic matter is converted to carbon dioxide and methane, *i.e.* biogas, by microorganisms in an oxygen-free environment. This process occurs naturally in swamps, sediments, rice fields, landfills and under controlled artificial conditions in anaerobic bioreactors [51-53]. Heterogeneous organic matter is broken down by the action of anaerobic microorganisms. These organisms utilize nitrogen, phosphorus, and other nutrients for their metabolism, but reduce organic nitrogen to organic acids and ammonia. The unutilized carbon, from the organic compounds, is mainly liberated in the form of methane, while some as carbon dioxide.

Anaerobic digestion has been used for treatment of wastewater and stabilisation of solid waste since latter part of the 19th century. In rural parts of China and India, simple reactor constructions have long been used to treat manure and agricultural wastes with the main purpose of recovering energy for cooking and lighting. Today, a number of different anaerobic digestion techniques are in use for different types of substrate and their development is still in progress [54].

Uncontrolled release of gaseous products of waste decomposition into the atmosphere contributes to global warming. Decomposition of each metric ton of solid waste could potentially release 50-110 m^3 of CO_2 and 90-140 m^3 of methane into the atmosphere. Methane gas traps 30 times more heat than CO_2 and contributes to 18% of the global warming. The energy value of 250 million metric tons of anthropogenic methane released worldwide in 1990 was equivalent to 16% of the energy consumption i.e. 14.2×10^{18} J/yr (13.5 Quads/yr) in the United States that year. Recovery of this methane as a biofuel could reduce global warming, firstly by preventing anthropogenic methane emission, and secondly by displacing fossil fuels. It is estimated that up to 20% reduction in global warming may be achieved by utilizing discarded biomass and wastes for the production of biofuels and chemicals [3].

Anaerobic degradation of carbohydrates, protein and fat from biowastes proceeds via hydrolysis, acidogenesis, acetogenesis and methanogenesis [55] in wet or dry biowaste fermentation systems. For wet fermentation, the dry matter content is adjusted to 8-16% by addition of process water, whereas for dry fermentation little process water is added or not added at all to the moist material. In an "optimized dry digestion system" the dry matter content was kept above 31% and the organic loading rate (OLR) was adjusted to 18.5 kg COD m^{-3} d^{-1} at 15.3 days retention time [56].

The complete anaerobic degradation of organic matter is a complex process involving a number of steps and microorganisms with different metabolic capacities [57]. In the first step, polymers such as lipids, proteins and polysaccharides are converted to monomers through hydrolysis. This degradation is performed by the action of extracellular enzymes produced by hydrolytic and fermentative bacteria. The monomers produced, *i.e.* amino acids, sugars and fatty acids, are then primarily fermented to volatile fatty acids, alcohols, hydrogen (H_2) and carbon dioxide (CO_2) by different groups of fermentative bacteria. In the third step, proton-reducing acetogenic bacteria produce acetate, hydrogen and carbon dioxide through anaerobic oxidation of the fermentation products. This step is endergonic under standard conditions and can only be performed if these organisms are operating in a syntrophic relationship with hydrogenotrophic methanogens [58,59]. Acetate can also be formed from hydrogen and carbon dioxide through the activity of hydrogen-oxidising acetogenic bacteria. The reverse reaction, *i.e.* the oxidation of acetate to CO_2 and H_2, can be performed under certain conditions by some acetogenic bacteria in syntrophic relationship with hydrogen-transforming methanogens [60-63]. Methanogenesis is the last step in the anaerobic digestion, with two main possibilities to produce methane. One involves the acetotrophic methanogens, which have acetate as substrate, whereas the hydrogenotrophic methanogens use the hydrogen and carbon dioxide produced [57].

During anaerobic digestion, not all of the organic matter is completely degraded and ends up in a residual product (digestate), which is also rich in inorganic nutrients. This makes the digestate an excellent complement to manure and commercial fertilizers on agricultural soils [64]. The digestate contains organic matter and plant nutrients (N, P, K and Mg) and these positively affect soil quality by improving the soil structure, increasing the water-holding capacity and stimulating the microbial activity [65,66]. The end result is not only an increase in soil quality, but also higher crop yields and better grain quality in comparison to unfertilised soil, or equivalent effects after application of artificial fertiliser [64].

However, it is important that the recycled digestates do not contain pathogens and/or chemical contaminants that may build up to deleterious levels in the soil. Unfortunately, different organic pollutants have been found in digestates. When considering the risks associated with the use of digestate as fertiliser, information on the character and the fate of the pollutant in anaerobic bioreactors and in soil is of importance [67]. Organic industrial wastes, manure and organic household wastes may contain a variety of organic pollutants, often with an aromatic structure [68]. In fact, dioxin-like compounds [69,70], polyaromatic hydrocarbons (PAH) [68], 2000), pesticides, PCBs [71], chlorinated paraffins [72], phthalates [68,73] and phenolic compounds [68] have been found in different digestates. The presence of these compounds can result in a digestate not suitable for use as a fertiliser on agricultural soils. These contaminants can originate from pesticide traces on fruit and vegetables or additives in plastic material, or may come from contamination in the collection and transport chain of the waste to the biogas plant [71,73].

Biowaste is known to contain pathogens such as Salmonella, Listeria and Campylobacter [74-76]. The biosecurity risk associated with using digested residue as fertilizer is hard to assess, but this risk cannot be neglected. It is of great importance that treatment in the biogas plants (BGPs) minimizes the survival of pathogens. Temperature is the most important factor when considering the reduction of pathogens in BGP, but there are also other factors involved. Different indicator bacteria are used to evaluate the hygienic treatment, but an indicator that is good enough to give an overall picture has not yet been found [77].

Pasteurization of biowaste at 70°C for an hour is an effective way of heat treatment to reduce most pathogens [78,79]. Pasteurization in a biogas plant can be performed batchwise or as a continuous process. The batch-wise method is preferred, as treatment time is more easily regulated and the pathogen reducing effect can be verified [80]. As many pathogens are difficult to detect and quantify, indicator bacteria are generally used to monitor the bacterial level and show the effect of disinfections [81]. An increased number of indicator bacteria indicate a possible increase in pathogens. *Escherichia coli* and *Enterococcus* spp. can be used as indicator bacteria [74,75].

In the beginning of the anaerobic digestion era, no heating or mechanical mixing was applied in bioreactors, resulting in low conversion efficiency and gas yield. Development of bioreactors operating at mesophilic (30-40ºC) and thermophilic (50-60 ºC) temperatures was a consequence of increased knowledge about reaction rates and growth optima for the anaerobic microorganisms active in the digestion process [82]. Anaerobic digestion at thermophilic temperature has a higher bioconversion rate and a shorter treatment time than digestion at lower temperature [83,84]. The increase in gas production at the higher temperature can compensate for the additional costs related to heating [85]. Another advantage of digestion at thermophilic temperatures is the comparatively stronger hygienisation effect, i.e. more efficient killing of pathogens present in the waste [77,80,83]. In comparison to thermophilic bioreactors, the mesophilic process requires less energy for heating and has a slower conversion rate for the organic material. This process is also commonly less affected by inhibitory effects of ammonia released during the mineralisation of proteins [86,87]. At present, the interest in anaerobic treatment under psychrophilic conditions (<20 ºC) is increasing due to its considerably lower heating costs [82]. New and modified bioreactors may successfully facilitate the use of this low temperature technique [88].

A major environmental benefit of the anaerobic digestion process is the production of biogas, a renewable energy source, which can be used as vehicle fuel, for heating and for electricity production. The use of fossil fuel can thereby be reduced, enabling CO_2 levels to be lowered in conformance with the Kyoto protocol [67].

CONCLUSION

Life is associated with waste generation and utilization of this resource for generation of useful product is a major challenge for biotechnology. Aerobic and anaerobic digestion may not have the glamour of genetic engineering, but hold immense promise for efficient waste management and future energy security.

It should be the aim of the microbiologists to know the diversity of microbes present in a certain system and their specific role in achieving the desired target. Although studies has been done on microbial ecology in garbage heaps, yet there are still many microflora which has not been isolated and identified but has potential for rapid and efficient decomposition of biowaste. Characterization of the microenvironment of compost heaps using microsensors will provide vital information on the microbial activities and ways to enhance their efficiency in decomposition of biological waste.

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